



TITLE:

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CITATION:

SUZUKI, KATSUYOSHI. HISTOCHEMICAL STUDIES ON CHOLINESTERASE IN PACINIAN CORPUSCLES : REPORT I. 日本外科宝函 1958, 27(5): 1055-1062

ISSUE DATE:

1958-09-01

URL:

<http://hdl.handle.net/2433/206696>

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HISTOCHEMICAL STUDIES ON CHOLINESTERASE IN PACINIAN CORPUSCLES

REPORT I

by

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(Received for publication June 23, 1958)

1. Introduction

In recent years, the metabolism of the acetylcholine, one of the chemical mediators, has been investigated in details by many authors. The enzymes connected with the formation and hydrolysis of the acetylcholine have also been studied by NACHMANSOHN and others.

Cholinesterase (ChE), the enzyme which hydrolyzes acetylcholine into choline and acetate, has more stable property than the acetylcholine in the tissue, so the former has been studied by many authors physiologically and enzymatologically.

Especially, histochemical demonstration made it possible to clarify the cellular localization of ChEs in the tissue, and the activity of ChE is considered to be in proportion to the stainability.

Histochemical methods have been reported by GOMORI (1945), KOELLE (1948) and RAVIN (1951). With these methods the localization of ChEs in various organs and tissues has been studied in details by KOELLE, RAVIN, TOYODA, UONO et al.

On the nervous system, KOELLE (1955) described high concentration of ChE localized in the cholinergic neurons, neuromuscular junctions and synapses of central nervous system of all species, and lower concentrations in all the adrenergic and sensory neurons examined. But there are few reports on ChE activity in the sensory receptors except the short reports of CSILLIK (1954), and STEIGLEDER (1957).

In this report, ChE activity in mesenteric Pacinian corpuscle and the change of ChE activity under various conditions was studied by KOELLE's method.

II. Materials and Methods

Pacinian corpuscles distributed to the mesenteries of the cat are macroscopically visible. Therefore the cat was used as experimental animal. 0.2g Ouropan Soda was given previous to the laparotomy of the cats, and they were anesthetised with ether during operation.

A) Pacinian corpuscles in normal state

Normal Pacinian corpuscles were dissected immediately after sacrificing the animal by bleeding.

B) Pacinian corpuscles under special conditions were dissected in 30-60 minutes after following treatments.

1) After obstructing the blood flow to Pacinian corpuscles by the ligature of A. and V. mesenterica ventralis.

2) After making the local blood congestion by the ligature of the V. mesenterica ventralis.

3) After making the local anemia by the ligature of the A. mesenterica ventralis.

4) After the administration of Diisopropyl fluorophosphate (DFP).

a) 1-2mg of DFP was administered intra-arterially through the A. mesenterica ventralis with the ligature of the V. mesenterica ventralis.

b) 3-4mg of DFP was administered intra-arterially through the A. mesenterica ventralis without ligating the V. mesenterica ventralis.

c) 3-8mg of DFP was administered intravenously through the V. cava caudalis.

20-30 of dissected Pacinian corpuscles were gathered to make a frozen piece of them as large as a rice corn, from which frozen sections were cut in $40-50\mu$ slice. Sections were placed on slides immediately. Histochemical demonstration of ChEs was carried out by KOELLE's modification method.

KOELLE's modification method

I. Frozen section were cut as soon as possible after removal, and placed immediately on slides.

2. As soon as the section was thawed and the excess moisture was evaporated, the slides were placed in the appropriate storage solutions for 30 minutes.

a. Sections to be stained for specific ChE activity and controls were placed in the storage solution of DFP (H_2O 4.5 cc, 40% Na_2SO_4 9.0 cc, $10^{-6}M$ DFP 1.5cc).

b. Sections to be stained for total ChE activity were placed in the storage solution B-D (H_2O 6.0cc, 40% Na_2SO_4 9.0cc).

c. Sections to be stained for non-specific ChE activity were stored in storage solution C (H_2O 4.5cc, 40% Na_2SO_4 10.5cc). Storage solution were kept in a water bath at $30-35^\circ C$.

3. Incubation solution (5-60 minutes at $38^\circ C$)

Composition of incubation media

Solution	Enzyme localized	BuThCh cc	Cu-Gl cc	H_2O cc	Mal cc	Na_2SO_4 cc	$MgCl_2$ cc	CuThCh	AThCh cc
B	Total & sp.ChE	—	0.6	2.1	1.5	9.0	0.6	Trace	1.2
C	Non-sp. ChE	1.2	0.6	0.6	1.5	10.5	0.6	//	—
D	Control	0.8	0.4	1.4	1.0	6.0	0.4	//	—

Reagents

1) Cu-Gl: Glycine 3.75g, $CuSO_4 \cdot 5H_2O$ 2.50g, q.s. 100.0cc

2) Mal: NaH maleate (J. W. Temple: J. Am. Chem. Soc. 51, 1754, 1929) 9.60g, 1 N NaOH 52.2cc q. s. 100.0cc.

3) Na_2SO_4 : 40% (W/V) Na_2SO_4 adjusted to pH 6.0 stored at $38^\circ C$.

4) $MgCl_2$: $MgCl_2$ 9.52g q. s. 100.0cc.

5) AThCh: 23mg Acetylthiocholine iodide, H_2O 1.2cc, 0.1 M $CuSO_4$ 0.4cc.

6) BuThCh: 43 mg Butyrylthiocholine iodide, H_2O 1.8cc, 0.1 M $CuSO_4$ 0.6cc centrifuged, supernatant decanted off and saved.

7) CuThCh: Acetylthiocholine 29.0mg, H₂O 1.0cc, 0.1M CuSO₄ 0.6cc centrifuged and filter. To filtrate and two 1.0cc washings, add 1.0cc each of reagent G1 (Glycin 1.88g IN KOH 2.0cc, q. s. 50.0cc) and 0.1M CuSO₄, alkalize to pH 9-10 with IN KOH.

Allow to stand several hours at room temperature until precipitation is complete. Collect precipitate by centrifugation, wash twice with small amounts of water.

4. Rinse solution 1. (5 minutes or longer) 20% Na₂SO₄ saturated with CuThCh.

5. Rinse solution 2. (1 minute) 10% Na₂SO₄ saturated with CuThCh.

6. Rinse solution 3. (1 minute) CuThCh saturated water.

7. Ammonium sulfide solution saturated with CuS. (20 seconds)

Half strength concentrated ammonium hydroxide solution saturated with H₂S, stored in refrigerator. Immediately before use, dilute 1:25, saturate with CuS by adding 0.1M CuSO₄ dropwise and filter.

8. Rinsed rapidly in CuS saturated water.

9. Fixed in CuS saturated 10% formalin.

ChE activity is stained yellowish brown.

In this report, sections were incubated in incubation solution during 60 minutes. MAYER's hematoxylin was used for counterstaining.

III. Result

A) Pacinian corpuscles in the mesentery of the cat in normal state. ChE activity was demonstrated in Pacinian corpuscles at central core. (Fig.)

B)

1) Pacinian corpuscles after the obstruction of blood supply or after making the congestion of blood, no change was observed in the ChE activity.

2) Pacinian corpuscles after the administration of DFP, ChE activity markedly decreased in proportion to the increased dosage of DFP, and when DFP was given more than a certain dosage ChE activity was demonstrated no more.

IV. Discussion

There are many reports that ChEs are localized in the cholinergic neurons, but the reports in the sensory corpuscle are rare. Especially no one studied it in the Pacinian corpuscle in the mesentery of the cat. The author succeeded in demonstrating the ChEs in the Pacinian corpuscle located in the mesentery of the cat. Pacinian corpuscles in mesentery of the cat varied from 0.46 to 1.55mm in length and from

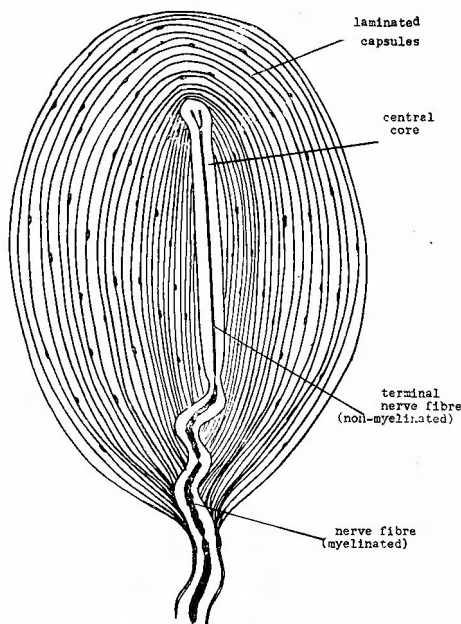


Fig. 1 Pacinian corpuscle

0.33 to 1.13mm in breadth (T. A. QUILLIAM and M. SATO). They consist of a central core which encloses a single non-myelinated terminal nerve fibre, and which is surrounded by laminated capsules like the sectioned onion. They are generally believed to serve as organs for pressure reception. They are visible macroscopically and exist numerously in the mesentery and easily available to experimental procedure. J. A. GRAY & M. SATO supposed that acetylcholine would be able to excite impulses at the terminals of sensory axons, but there are no evidence that acetylcholine plays physiological role there.

Therefore histochemical demonstration of ChEs in Pacinian corpuscle, one of the typical sensory receptors, may be very important, because the localization of ChEs in Pacinian corpuscle limited to the central core will be indirect evidence that acetylcholine is released to the central core by certain stimuli. Furthermore, the ChEs found in the mesenteric Pacinian corpuscles may suggest that acetylcholine can be a chemical mediator on some visceral sensory nerve ending.

ChEs are divided into specific and non-specific by nature. Specific ChE existed in nervous system and muscles hydrolyzes acetylcholine specifically, and non-specific ChE existed in liver and serum hydrolyzes acetylcholine, various cholinesters or non-choline esters. In this report, the ChEs localized in central core of Pacinian corpuscle were demonstrated, but it was not decided whether they are specific or non-specific. Further investigations are necessary to determine this problem.

The table showing ChE activity in various condition only indicates the degree of stainability, but not the quantitative measurement of ChE.

Local anemia and blood congestion, artificially produced by ligating the A. and V. mesenterica ventralis, may act as a stimulus for Pacinian corpuscle, but the concentration of ChEs is not changed after these procedures.

These facts agree with the presence of ChEs in excess in tissues, which

b)ChE activity after intraarterial administration of DFP

Animal No.	DFP mg	V.mesent. ligated.	ChE activity
9	1.0	+	##
10	"	+	+
11	1.5	+	+
13	1.0	+	+
17	2.0	+	÷
22	"	+	-
12	3.0	-	+
14	4.0	-	÷

Table

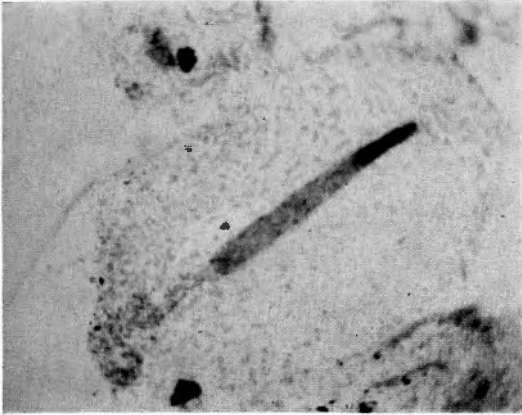
a)ChE activity after ligation of vessels

Animal No.	Ligature	ChE activity
2	A.& V.mesent.	##*
3	"	##
4	V.mesent.	##
6	"	##
7	"	##
5	A.mesent.	##
15	"	##
16	"	##

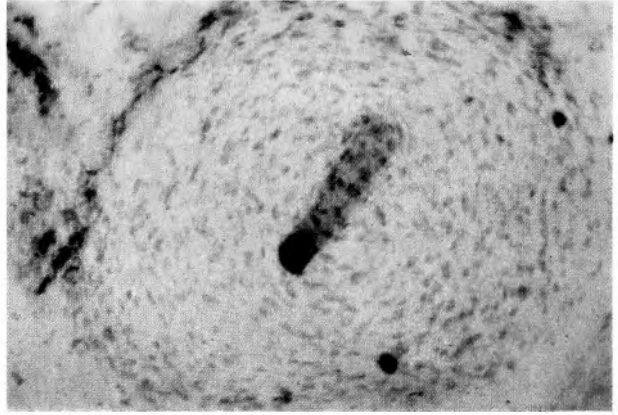
c)ChE activity after intravenous administration of DFP

Animal No.	DFP mg	Body weight Kg	Dosage mg/kg	ChE activity
18	4.0	2.0	2.0	÷
19	"	2.6	1.5	+
20	8.0	3.7	2.2	-
21	6.0	3.5	1.7	÷

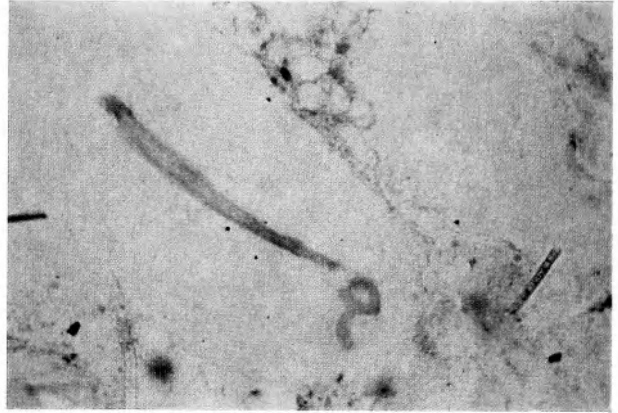
*Normal ChE activity in Pacinian corpuscle is expressed with ##

Fig. 2 ChE activity in the central core of Pacinian corpuscle.i) longitudinal sections ($\times 100$)ii) transvers section ($\times 200w$)

a) with counterstaining by hematoxylin



with counterstaining by hematoxylin



b) without counterstaining

amounts in some nerves to about 10 to 12 times the concentration necessary for normal function.

DFP is an irreversible anti-ChE compound, and their pharmacologic effects are due to the inhibition of ChE activity in the tissue, which results in cholinergic effects by the accumulation of acetylcholine in the effector organs. Its 50% fatal dosis (LD_{50}) of the cat is 1.65 ± 0.03 mg/kg by intravenous administration (HORTON: J. Pharm. & Exp. Therap. 87, 414, 1946).

KOELLE reported that in the brain of animals dead by LD_{50} of DFP ChE activity was not demonstrated, but in survivals ChE activity were reduced to 10-20% of normal animals.

NACHMANSOHN and FELD (1947) stated that absence of ChEs in brain always coincides with death.

In this report, ChE activity after the DFP administration decreased in proportion to the dosage of DFP, and even when DFP was given beyond the lethal dose intravenously, the animals could be alived, but showing no ChE activity in Pacinian corpuscle.

V. Summary

1) By KOELLE's histochemical method remarkable ChE activity was demonstrated in central core of Pacinian corpuscle.

2) By the obstruction of blood supply or by making the congestion ChE activity in the Pacinian corpuscles gave no remarkable change.

3) Administration of DFP reduced the ChE activity in Pacinian corpuscles in proportion to the dosage.

I am very grateful to Assist. Prof. Ch. Kimura who gave me constant help and kind guidance throughout this work.

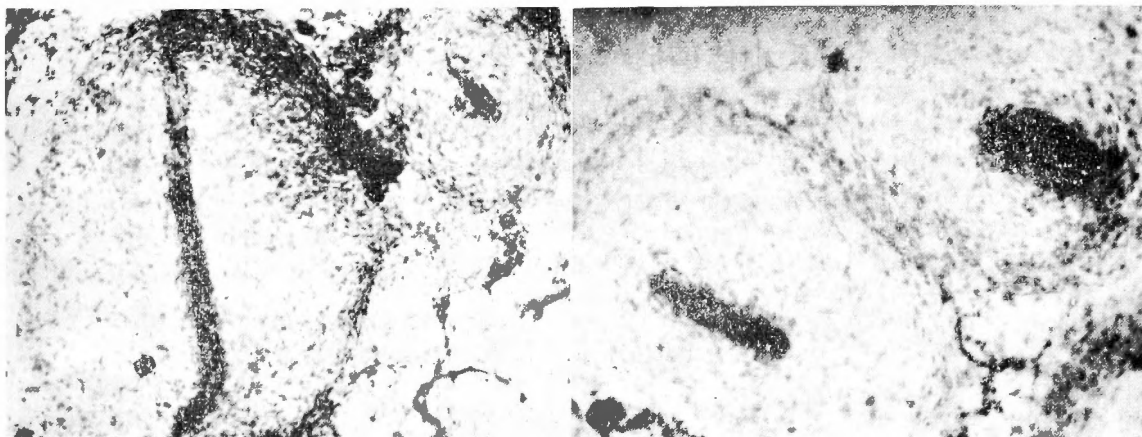


Fig. 3 Multiple sections of Pacinian Corpuscle showing in a slice. ($\times 100$)

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和 文 抄 録

パチニー氏小体に於けるコリンエステラーゼの 組織化学的研究 第1報

京都大学医学部外科学教室第2講座

(指導: 青柳安誠教授) 大阪北通信病院外科 (部長 岡村 正博士)

鈴 木 克 義

Koelle の方法を用いて、猫の腸間膜に分布するパチニー氏小体に於けるコリンエステラーゼの組織化学的研究を行い、次の結論を得た。

1) パチニー氏小体の内楕部に局限して、著明なコリンエステラーゼの活性を認めた。

この事は、圧覚受容器と云われるパチニー氏小体の内楕部に於て無髄神経末端部にアセチルコリンが作用する事を暗示するものである。

2) パチニー氏小体の分布する領域の血管を結紮し

て、局所の貧血或は鬱血を起しても、コリンエステラーゼの活性には特に変化を認めなかつた。

3) パチニー氏小体を支配する動脈からか或は、全身性に下空静脈から DFP を投与すると、パチニー氏小体のコリンエステラーゼ活性は著明に減少した。減少の割合はほぼ投与量に比例し、一定量以上を投与すればコリンエステラーゼを証明する事が出来なかつた。